Structure and function of components of the bacterial phosphotransferase system

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Summary

The reversible phosphorylation of proteins is a cardinal regulatory mechanism in living cells. Amongst others it is crucial in cell growth, differentiation, metabolism, virulence, and the transformation of normal cells to cancer cells. In bacteria three evolutionary distinct phosphorylating systems exist: the classical ATP-dependent protein kinases, the sensor-kinase/response-regulator systems (or two-component systems), and the phosphoenol-pyruvate-dependent phosphotransferase system (PTS). The PTS differs from the other systems in the way that it combines carbohydrate-uptake with the regulation of many cellular processes, which vary from the regulation of gene transcription to chemotaxis towards its substrates. The PTS is normally composed of two general proteins, enzyme I (E_I) and the histidine-containing protein (HPr), and a carbohydrate-specific complex enzyme II (E_{II}). E_{II}s usually consist of two cytoplasmic domains, IIA and IIB, and a transmembrane channel IIC. The energy to drive carbohydrate-transport across the cytoplasmic membrane of the bacterium is provided by phosphoenolpyruvate, which is used as a phosphoryl group donor in the autophosphorylation of E_I. *Via* HPr and IIA, the phosphoryl group is transferred to IIB, which in its phosphorylated state triggers rapid transport of the carbohydrate by IIC and subsequently phosphorylates the transported carbohydrate. The phosphorylated carbohydrate is released in the cytoplasm where it can be used as an intermediate in the bacterial metabolism.

This thesis describes the determination of the crystal structures of three components of the *Escherichia coli* PTS: the cellobiose-specific IIB enzyme (IIB^{cel}), the mannitol-specific IIA domain (IIA^{mtl}), and the regulatory $IIA^{nitrogen}$. These three structures, together with the already available structural information, provide a firm basis to elucidate the mechanism of PTS-dependent carbohydrate transport in molecular detail, and contribute to our understanding of the complex nature of (bacterial) signalling systems/signal transduction.

Chapter 1 gives an overview of the three phosphorylating systems occurring in bacteria, followed by a detailed description of the various components of the PTS in relation to the available structural information. Furthermore, it provides a description of the regulatory functions of the PTS.

Chapter 2 describes the crystallisation of the IIB enzyme of the cellobiose-specific enzyme II (IIB^{cel}). Crystals of Cys10Ser mutant IIB^{cel} could be grown with the hanging drop method of vapour diffusion, using both streak-seeding and macroseeding techniques. The addition of

benzamidine/HCl and 2-propanol was essential to obtain single crystals, instead of crystals with a layered structure.

Chapter 3 presents the crystal structure of IIB^{cel}, which was determined by multiple isomorphous replacement. It consists of a central four-stranded parallel β-sheet, flanked by α-helices on both sides. The phosphorylation site, Cys 10, is located on the C-terminal end of the first β-strand. The structure of IIB^{cel} is remarkably similar to the fold of the low molecular weight protein tyrosine phosphatases (LMW PTPases), a class of mammalian signalling proteins, which, like IIB^{cel}, feature a phosphocysteine intermediate in their reaction mechanism. A comparison of the structures of IIB^{cel} and bovine liver LMW PTPase provided insight into the mechanism of the phosphoryl transfer reactions in which IIB^{cel} is involved. Another class of proteins structurally similar to IIB^{cel} is the class of periplasmic sugar-binding proteins. A structural comparison of IIB^{cel} with the arabinose binding protein revealed a possible interaction-site for the IIC^{cel}-cellobiose complex. Surprisingly, the functionally similar IIB domain of the glucose-specific EII (IIB^{glc}) has an entirely different fold. This explains why the carbohydrate-specific components of different EII-families cannot complement each other.

Chapter 4 focuses on the structure determination of the IIA domain of the mannitol-specific enzyme II. The structure was determined by multiple wavelength anomalous dispersion on a selenovariant of IIA^{mtl}, followed by a combination of solvent flattening, histogram matching, and four-fold averaging. It consists of a five-stranded mixed β-sheet flanked by three α-helices on one side of the sheet and two on the other side. The phosphorylation site, His 65, is located at the end of the third β-strand. His 65 is flanked by the conserved Arg 49 on one side, and His 111 on the other side. In the four independent IIA^{mtl} molecules Arg 49 and His 111 adopt two different conformations, which might represent different states of the active site, required for the different phosphoryl transfer reactions in which IIA^{mtl} is involved. Using a solution structure of phosphorylated HPr, and a combination of molecular modelling and NMR binding experiments, structural models of the HPr-IIA^{mtl} interaction complex were generated. A comparison of our best HPr-IIA^{mtl} complex with models of HPr in complex with other IIA enzymes showed that the overall interaction mode between the two proteins is similar.

Chapter 5 presents the structure of IIA^{ntr}. IIAntr, which is homologous to the IIA proteins of the mannitol-fructose E_{II} family, negatively regulates the secondary sigma factor σ^{54}, by an unknown mechanism. As IIA^{ntr} can be phosphorylated by HPr and the HPr-homologue NPr this mechanism is likely to involve the phosphorylation state of IIA^{ntr}. The structure of IIA^{ntr}

was solved by multiple isomorphous replacement. It is extremely similar to the fold of IIA^{mtl}. Nevertheless, significant differences exist in the details of their active sites.

Chapter 6 discusses these differences in relation to the respective functions of IIA^{mtl} and IIA^{ntr}. Most pronounced is the different location of the second conserved histidine residue. In IIA^{mtl}, this residue (His 111) is located in the active site close to the phosphorylation site, which substantiates the proposal that it plays a role in the phosphoryl transfer from IIA^{mtl} to IIB^{mtl}. In contrast, the equivalent His 120 in IIA^{ntr}, is not found in the active site. Instead, it points into the solvent on the other side of the protein. The position of His 120 makes a direct role in phosphoryl transfer from IIA^{ntr} to another protein analogous to phosphoryl transfer from IIA^{mtl} to IIB^{mtl} unlikely. It conservation among IIA^{ntr} enzymes, however, suggests an important function for this residue. As His 120 is located on the surface of the molecule it might have a role in the recognition of a target protein of IIA^{ntr}.

The rapid advance in the structural characterisation of the PTS has added a new dimension to PTS research, as now some of the PTS-questions can be addressed from a structural perspective. Unfortunately, a number of questions remain to be answered, many of them due to a lack of structural information on the membrane-bound IIC domain. The structure determination of IIC domains will therefore be one of the most challenging goals in future PTS-research.